

## **LISTING OF THE CLAIMS**

1. (Currently amended) A microfluidic device for processing a cell-containing microdroplet, comprising:
  - a channel,
  - a lysing zone connected to the channel,
  - ~~a thermopneumatic~~ an actuator connected to the channel only upstream of the lysing zone and configured to create a difference between an upstream pressure and a downstream pressure acting on the cell-containing microdroplet to move the microdroplet at least partially into the lysing zone,
  - a vent connected to the channel upstream of the lysing zone and configured to reduce the pressure difference to stop the cell-containing microdroplet in a lysing position with respect to the lysing zone, and a lysing mechanism to release intracellular material from cells within the cell-containing microdroplet stopped within the lysing zone
  - ~~wherein the thermopneumatic actuator is connected.~~
2. (Original) The microfluidic device of claim 1, wherein the cell-containing microdroplet comprises cells entrained in a liquid.
3. (Original) The microfluidic device of claim 2, wherein actuation of the lysing mechanism subjects cells in the lysing zone to an electric field sufficient to release the intracellular material.
4. (Original) The microfluidic device of claim 3, wherein actuation of the lysing mechanism subjects substantially all of the cells in the lysing zone to the electric field to prepare a microdroplet comprising the released intracellular material.
- 5-8. (Cancelled)
9. (Previously presented) The microfluidic device of claim 13, wherein the reduced wetting material is hydrophobic.
- 10-11. (Cancelled)

12. (Currently amended) The microfluidic device of claim [[2,]] 1, wherein the vent is configured to position a downstream portion of the cell-containing microdroplet in the lysing position.

13. (Previously presented) The microfluidic device of claim 12, comprising a reduced wetting material configured to prevent the microdroplet from contacting the vent.

14. (Previously presented) The microfluidic device of claim 13, further comprising a valve to selectively obstruct or allow passage of gas between the reduced wetting material and the vent.

15. (Cancelled)

16. (Currently amended) A microfluidic device for processing a cell-containing fluid, comprising:

a channel;

a lysing zone connected to the channel;

a lysing mechanism to release intracellular contents from cells within the lysing zone;

a first gas actuator connected to the channel only upstream of the lysing zone and configured to move an amount of a cell-containing fluid downstream at least partially into the lysing zone;

a positioning element located downstream of the lysing zone and configured to inhibit downstream movement of the cell containing fluid, thereby positioning at least some of the cell containing fluid in a lysing position with respect to the lysing zone; and

a ~~thermopneumatic~~ second gas actuator disposed upstream from the positioning element to provide a gas pressure sufficient to (a) prepare a microdroplet comprising intracellular contents released from cells of the cell-containing fluid within the lysing zone, the microdroplet having a length substantially equal to a distance between the gas actuator and the positioning element and (b) move the microdroplet downstream of the lysing zone and past the positioning element.

17. (Original) The microfluidic device of claim 16, wherein the fluid of the cell-containing fluid is a liquid.

18. (Original) The microfluidic device of claim 17, wherein actuation of the lysing mechanism subjects at least some cells in the lysing zone to an electric field sufficient to release the intracellular contents of the cells.
19. (Original) The microfluidic device of claim 18, wherein the microdroplet is essentially free of cells that have not been subjected to the electric field.
20. (Cancelled)
21. (Previously presented) The microfluidic device of claim 17, wherein the distance between the gas actuator and the positioning element is configured such that the microdroplet comprises less than about 90 percent of the amount of the cell-containing fluid.
22. (Currently amended) The microfluidic device of claim 17, wherein the device comprises a substrate, and wherein the lysing zone and first gas actuator are integral with the substrate.
23. (Currently amended) The microfluidic device of claim 22, wherein the first gas actuator comprises a heat source to heat an amount of gas thereby increasing a pressure of the gas.
24. (Cancelled)
25. (Previously presented) The microfluidic device of claim 17, wherein the positioning element increases a surface tension of a downstream portion of the cell-containing fluid to thereby inhibit downstream movement of the cell-containing fluid.
26. (Previously presented) The microfluidic device of claim 17, wherein the device comprises a vent to substantially equalize a gas pressure upstream of the cell-containing fluid with a gas pressure downstream of the cell-containing fluid when the cell-containing fluid is in the lysing position to thereby inhibit downstream movement of the cell-containing fluid downstream from the lysing position.
27. (Previously presented) A microfluidic method for processing a cell-containing liquid microdroplet, comprising:  
propelling a microdroplet toward a lysing mechanism by increasing a gas pressure

upstream of the microdroplet,

venting gas from upstream of the microdroplet to reduce the upstream pressure and stop the cell-containing microdroplet in a lysing position with respect to a lysing mechanism of a microfluidic device; and

actuating a lysing mechanism to release intracellular material from cells of the stopped cell-containing microdroplet.

28. (Currently amended) The microfluidic method of claim 27, comprising increasing a surface tension of a downstream surface of the microdroplet.

29. (Currently amended) The microfluidic method of claim 28, wherein the increasing [[step]] comprises contacting the downstream surface of the microdroplet with a hydrophobic material.

30. (Currently amended) The microfluidic method of claim 28, wherein the increasing [[step]] comprises increasing a radius of curvature of the microdroplet.

31. (Cancelled)

32. (Original) The microfluidic method of claim 27, wherein the actuating step comprises subjecting cells of the microdroplet to an electric field sufficient to release intracellular contents from the cells.

33. (Previously presented) A microfluidic method for processing a cell-containing liquid, comprising:

introducing the cell-containing liquid to a lysing zone of a microfluidic device;  
inhibiting liquid of the cell-containing liquid from exiting the lysing zone, then actuating the lysing mechanism to release intracellular contents from cells of the cell-containing liquid within the lysing zone;

and then providing a gas pressure sufficient to separate a first portion of the cell-containing liquid located within the lysing zone from a second portion of the cell-containing liquid located upstream of the lysing zone to prepare a microdroplet comprising intracellular contents released from cells of the cell-containing liquid within the lysing zone.

34. (Cancelled)

35. (Original) The microfluidic method of claim 33, wherein actuating the lysing mechanism subjects at least some cells within the lysing zone to an electric field sufficient to release the intracellular contents of the cells.

36. (Original) The microfluidic device of claim 35, wherein the microdroplet is essentially free of cells that have not been subjected to the electric field.

37. (Previously presented) The microfluidic device of claim 33, wherein the step of providing the gas pressure moves the microdroplet to a location downstream of the lysing zone.

38. (Withdrawn) A microfluidic substrate for processing fluids comprising: a lysing module for releasing intracellular material from cells within the sample to thereby forming a lysed sample, a microdroplet formation module for forming a first microdroplet of fluid from the lysed sample, a mixing module for mixing said first microdroplet with a second microdroplet comprising a reagent to form a third microdroplet, and an amplification module for amplifying intercellular material within said third microdroplet.

39. (Currently amended) A microfluidic device for processing a cell-containing sample material, the device comprising:

- a sample passage;

- a lysing zone in communication with the sample passage;

- a ~~first thermopneumatic~~ first thermopneumatic gas actuator to move an amount of cell-containing sample liquid along the sample passage toward the lysing zone;

- a second ~~thermopneumatic~~ thermopneumatic gas actuator to move only a portion of the amount of the cell-containing sample liquid downstream of the lysing zone upon lysis of cells of the cell-containing sample liquid; and

- a plurality of valves, at least one of the valves located upstream of the lysing zone and at least one of the valves located downstream of the lysing zone, wherein, the valves, when in a closed state, inhibit the passage of material between the lysing zone and other portions of the microfluidic device.

40. (Currently amended) A method for lysing cells, comprising:

- ~~thermopneumatically~~ thermopneumatically moving a cell-containing liquid within a microfluidic device in response to a change in a pressure of a gas, the microfluidic device comprising a

lysing zone, a first passage upstream of the lysing zone, and a second passage downstream of the lysing zone, the cell-containing liquid being moved along the first passage and into the lysing zone;

inhibiting liquid of the cell-containing liquid from moving downstream of the lysing zone along the second passage; and

after inhibiting downstream movement of the liquid, lysing cells of the cell-containing liquid within the lysing zone.

41. (Previously presented) The method of claim 40, wherein the inhibiting comprises equalizing a pressure acting on the cell-containing liquid to prevent the cell-containing liquid from moving downstream of the lysing chamber, at least some of the cell-containing liquid stopping within the lysing zone.

42. (Previously presented) The method of claim 40, wherein the inhibiting comprises contacting a downstream boundary of the cell-containing liquid with a reduced wetting material disposed within the second passage.

43. (Currently amended) The method of claim 40, further comprising, after lysing cells of the cell-containing liquid, actuating a ~~thermopneumatic~~ gas actuator to separate a first portion of the cell-containing fluid from a second portion of the cell containing fluid.

44. (Currently amended) The method of claim 43, wherein the actuating the ~~thermopneumatic~~ gas actuator moves the first portion of the cell-containing fluid along the downstream passage.